

That which is claimed is:

1. A nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence.

2. A nucleic acid construct according to claim 1 wherein said germline-specific promoter is the protamine 1 gene promoter, the protamine 2 gene promoter, the spermatid-specific promoter from the c-kit gene, the sperm-specific promoter from angiotensin-converting enzyme, oocyte specific promoter from the ZP1 gene, oocyte specific promoter from the ZP2 gene, or oocyte specific promoter from the ZP3 gene.

3. A nucleic acid construct according to claim 1 wherein said germline-specific promoter is the LAT52 gene promoter from tomato, the LAT56 gene promoter from tomato, the LAT59 gene promoter from tomato, the pollen-specific promoter of the Brassica S locus glycoprotein gene, or the pollen-specific promoter of the NTP303 gene.

4. A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes Cre recombinase.

5. A nucleic acid construct according to claim 4 wherein said construct is ProCre, comprising the protamine 1 gene promoter operatively associated with Cre recombinase.

6. A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes FLP recombinase.

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7. A nucleic acid construct according to claim 6 wherein said construct is ProFLP, comprising the protamine 1 gene promoter operatively associated with FLP recombinase.

8. A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes the R gene product of *Zygosaccharomyces*.

9. A nucleic acid construct according to claim 8 wherein said construct is ProR, comprising the protamine 1 gene promoter operatively associated with the R gene product of *Zygosaccharomyces*.

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10. A nucleic acid construct comprising a conditional promoter operatively associated with a recombinase coding sequence.

11. A nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

12. Embryonic stem cells containing a nucleic acid construct according to claim 1.

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13. Embryonic stem cells according to claim 12 wherein the genome thereof comprises a transcriptionally active selectable marker flanked by two recombination target sites.

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14. Embryonic stem cells according to claim 13 wherein the recombinase encoded by the recombinase coding sequence operatively associated with a germline-specific promoter is selective for the recombination target sites flanking said selectable marker.

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15. Embryonic stem cells according to claim 13 further comprising one or more of:

a nucleic acid fragment flanked by two recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising a conditional promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

16. Embryonic stem cells containing a nucleic acid construct according to claim 2.

17. Embryonic stem cells containing a nucleic acid construct according to claim 3.

18. Embryonic stem cells containing a nucleic acid construct according to claim 4.

19. Embryonic stem cells containing a nucleic acid construct according to claim 5.

20. Embryonic stem cells containing a nucleic acid construct according to claim 6.

21. Embryonic stem cells containing a nucleic acid construct according to claim 7.

22. Embryonic stem cells containing a nucleic acid construct according to claim 8.

23. Embryonic stem cells containing a nucleic acid construct according to claim 9.

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24. Embryonic stem cells containing a nucleic acid construct according to claim 10.

25. Embryonic stem cells according to claim 24 wherein the genome thereof comprises a transcriptionally active selectable marker flanked by two recombination target sites.

26. Embryonic stem cells containing a nucleic acid construct according to claim 11.

27. Embryonic stem cells according to claim 26 wherein the genome thereof comprises a transcriptionally active selectable marker flanked by two recombination target sites.

28. A method for excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 13, said method comprising:  
passaging the genome derived from said embryonic stem cells through gametogenesis.

29. A method according to claim 28 wherein said genome is passaged through spermatogenesis.

30. A method according to claim 28 wherein said genome is passaged through oogenesis.

31. A method according to claim 28 wherein said embryonic stem cells further comprise one or more of:

a nucleic acid fragment flanked by two recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising a conditional promoter operatively associated with a recombinase coding sequence, or

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a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

32. A method for the production of recombinant alleles, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells according to claim 10, and

passaging the genome derived from said embryonic stem cells through gametogenesis.

33. A method according to claim 32 wherein said nucleic acid fragment comprises an essential portion of a gene of interest.

34. A method according to claim 32 wherein said nucleic acid fragment is introduced by homologous recombination, random insertion, retroviral insertion, or site specific-mediated recombination.

35. A method for the production of recombinant alleles, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells according to claim 13, and

passaging the genome derived from said embryonic stem cells through gametogenesis.

36. A method according to claim 35 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising a conditional promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

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<sup>27</sup><sub>31</sub>. A method according to claim <sup>26</sup><sub>36</sub> wherein the recombinase encoded by said second construct is expressed in response to inducing conditions.

<sup>28</sup><sub>38</sub>. A method according to claim <sup>26</sup><sub>36</sub> wherein the recombinase encoded by said second construct is expressed in a tissue selective manner.

39. A method according to claim 35 wherein the recombination target sites flanking said nucleic acid fragment are recognized by a recombinase which is expressed under the control of a conditional promoter or a tissue specific promoter.

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40. A method for the production of recombinant alleles, said method comprising:

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introducing at least one recombinase responsive construct into embryonic stem cells according to claim 10, wherein said construct(s) comprise(s) a nucleic acid fragment and a selectable marker, wherein said selectable marker is flanked by a first pair of recombination target sites, and wherein said nucleic acid fragment is flanked by a second pair of recombination target sites,

passaging the genome derived from said embryonic stem cells through gametogenesis.

41. A method according to claim 40 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germline-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of a conditional promoter or a tissue specific promoter.

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42. A method according to claim 40 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising a conditional promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

43. A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombination target site, and

wherein at least one of said segments contains at least two recombination target sites,

said method comprising:

introducing said individual inactive gene segments into an embryonic stem cell according to claim 10, thereby providing a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said stem cells through gametogenesis.

44. A method for the generation of recombinant livestock, said method comprising:

combining embryonic stem cells that include a nucleic acid construct according to claim 1 with host pluripotent ES cells derived from early preimplantation embryos, and

introducing these combined embryos into a host female and

allowing the derived embryos to come to term.

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45. A method for the generation of recombinant plants, said method comprising transforming plant zygotes with nucleic acid constructs according to claim 1 and allowing the zygote to develop.

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